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=> s fluorescen? or h1.671.768.369/ct or (reemiss? or re(w)emiss? or reemit?
or re(w)emit? or excit?)(2a)light or fluorescen?(2a)(emiss? or emit? or
excit?)

L1       208356 FILE MEDLINE
L2       264187 FILE CAPLUS
L3       163753 FILE BIOSIS
L4       110976 FILE EMBASE
L5        52547 FILE WPIDS
L6        36127 FILE JICST-EPLUS
'CT' IS NOT A VALID FIELD CODE
L7          116 FILE CONF
L8        15012 FILE NTIS

TOTAL FOR ALL FILES
L9       851074 FLUORESCEN? OR H1.671.768.369/CT OR (REEMISS? OR RE(W) EMISS?
                OR REEMIT? OR RE(W) EMIT? OR EXCIT?)(2A) LIGHT OR
FLUORESCEN?(2A
                )(EMISS? OR EMIT? OR EXCIT?)

=> s l9 and (lipid membrane? or liposome? or h1.181.529.530.517/ct)

L10        3637 FILE MEDLINE
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L11        4614 FILE CAPLUS
L12        2626 FILE BIOSIS
L13        2216 FILE EMBASE
L14         171 FILE WPIDS
L15         323 FILE JICST-EPLUS
'CT' IS NOT A VALID FIELD CODE
L16           0 FILE CONF
L17          43 FILE NTIS

TOTAL FOR ALL FILES
L18       13630 L9 AND (LIPID MEMBRANE? OR LIPOSOME? OR H1.181.529.530.517/CT)

=> s l18 and (lipophilic or nanocrystal?)

L19          75 FILE MEDLINE
L20         128 FILE CAPLUS
L21          56 FILE BIOSIS
L22          52 FILE EMBASE
L23           9 FILE WPIDS
L24           3 FILE JICST-EPLUS
L25           0 FILE CONF
L26           0 FILE NTIS

TOTAL FOR ALL FILES
L27         323 L18 AND (LIPOPHILIC OR NANOCRYSTAL?)

=> s (label? or detect?) and l27

L28          29 FILE MEDLINE
L29          43 FILE CAPLUS
L30          23 FILE BIOSIS
L31          24 FILE EMBASE
L32           6 FILE WPIDS
L33           1 FILE JICST-EPLUS
L34           0 FILE CONF
L35           0 FILE NTIS

TOTAL FOR ALL FILES
L36         126 (LABEL? OR DETECT?) AND L27

=> s l36 and (cell membrane or morphol? or photodetect? or filter or ccd
camera or microscop? or endoscop? or (fiber or fibre)(w)optic? or computer)

L37          20 FILE MEDLINE
L38          22 FILE CAPLUS
L39          12 FILE BIOSIS
L40          14 FILE EMBASE
L41           1 FILE WPIDS
L42           1 FILE JICST-EPLUS
L43           0 FILE CONF
L44           0 FILE NTIS

TOTAL FOR ALL FILES
L45          70 L36 AND (CELL MEMBRANE OR MORPHOL? OR PHOTODETECT? OR FILTER
OR
               CCD CAMERA OR MICROSCOP? OR ENDOSCOP? OR (FIBER OR FIBRE)(W)
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OPTIC? OR COMPUTER)

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PROCESSING COMPLETED FOR L45
L46 32 DUP REM L45 (38 DUPLICATES REMOVED)

=> d 1-32 cbib abs;s lipophilic(l)nanocrystal?(l)fluorescen?

L46 ANSWER 1 OF 32 MEDLINE
2000400289 Document Number: 20329608. Membrane lipid composition of Bacillus
stearothermophilus as affected by **lipophilic** environmental pollutants: an approach to membrane toxicity assessment. Donato M M; Jurado A S; Antunes-Madeira M C; Madeira V M. (Centro de Neurociencias, Universidade de Coimbra, 3000 Coimbra, Portugal.) ARCHIVES OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY, (2000 Aug) 39 (2) 145-53. Journal code: 6YD. ISSN: 0090-4341. Pub. country: United States.
Language:
English.
AB The thermophilic eubacterium Bacillus stearothermophilus is used as a model to identify membrane perturbing effects of **lipophilic** compounds. A parallelism has been established between the toxicity of the organochlorine insecticide DDT and its metabolite, DDE, in bacterial growth and the effects on cell functions and physical perturbations induced at the membrane (Donato et al. 1997a, Arch Environ Contam Toxicol 33:109-116; Donato et al. 1997b, Appl Environ Microbiol 63:4948-495). In the present work, the use of B. stearothermophilus as a model of screening for chemical toxicity has been implemented. Because the regulation of the lipid composition of the membrane is a common strategy in response to adverse growth conditions, we studied the effects of DDE on the lipid composition and the consequent alterations of membrane physical properties in comparison to the parental compound DDT. As expected, different adaptation responses were induced by the compounds, being DDT more effective as compared with DDE. Collected data are consistent with the stronger perturbations induced by DDT on growth and membrane functions. It is concluded that the membrane lipid composition of the bacterium is a very sensitive criterium to **detect** membrane-mediated toxic effects at low concentrations of **lipophilic** xenobiotics.

L46 ANSWER 2 OF 32 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 2000-105068 [09] WPIDS
AB US 6004536 A UPAB: 20000218
NOVELTY - Cyanine dye derivatives (I) are new.
DETAILED DESCRIPTION - Cyanine dye derivatives of formula (I) are new.
Q = (CH=CH)nCH optionally substituted by T;
n = 0-3;
T = F, Cl or 1-6C alkyl;
R1, R2 = 7-30C hydrocarbon optionally substituted by T1 and

optionally incorporating a 5- or 6-membered unsaturated hydrocarbon ring;

T1 = F, Cl or 1-6C alkoxy;

X1, X2 = O, S or CR3R4;

R3, R4 = 1-6C alkyl; or

R3+R4 = 5- or 6-membered saturated ring;

R5-R12 = H, F, Cl, Br, I, cyano, sulpho, phenyl, sulphophenyl, polysulphophenyl, carboxy, amino, ammonium, methylbenzamido, LA1 or 1-22C alkyl (optionally substituted by at least one F, Cl or OR13); or

two adjacent R5-R12 = fused benzo;

R13 = H or 1-6C alkyl;

L = single covalent bond or 1-20 atom linkage comprising C, N, O and S and any combination of ether, thioether, amine, ester, carboxamide, sulphonamide and hydrazide bonds, single, double, triple and aromatic C-C bonds, aromatic and heteroaromatic bonds;

A1 = reactive group; and

M = counterion or absent;

provided that at least one of R5-R12 = phenyl, sulpho, sulphophenyl or polysulphophenyl.

An INDEPENDENT CLAIM is also included for a method of staining **lipophilic** structures in cells comprising combining cells with a cyanine dye of formula (Ia) so that (Ia) selectively accumulates in the **lipophilic** structures.

R5a-R12a = H, F, Cl, Br, I, cyano, sulpho, phenyl, sulphophenyl, polysulphophenyl, carboxy, amino, ammonium, methylbenzamido, 1-22C alkyl (optionally substituted by at least one F, Cl or OR13), methylbenzamido, chloromethylbenzamido, maleimidyl benzamido, maleimidyl alkylamido, azidobenzamido or azidoperfluoro benzamido; or

two adjacent R5a-R12a = fused benzo;

provided that no more than one of R5a-R8a and no more than one of R9a-R12a is methylbenzamido, chloromethylbenzamido, maleimidyl benzamido, maleimidyl alkylamido, azidobenzamido or azidoperfluoro benzamido.

USE - (I) are useful for staining lipid structures particularly lipid structures of biological cells, tissues, **liposomes** and lipoproteins. Reactive (I) are also useful for preparing dye-conjugates of organic compounds, including conjugates of specific binding pairs so that membrane staining ability of the dye is conferred onto the dye-conjugate, allowing anchoring of an organic substance to a membrane. The **fluorescence** of (I) allows precise monitoring of the location of the dye conjugate.

ADVANTAGE - (I) are well-retained in lipid structures. Sulphonated and chemically reactive (I) resist extraction with organic solvents typically used in histochemistry for cell permeabilization before the use of secondary **detection** agents. (I) exhibit bright **fluorescence** in **cell membranes** and certain (I) (not specified) do not require the presence of an osmotic regulating agent to achieve sufficient loading of cells.

DESCRIPTION OF DRAWING(S) - The drawing shows the spectrum obtained when 1,1'-dioctadecyl-6,6'-di(4-sulphophenyl)-3,3,3',3'-tetramethylindocarbocyanine (I') (Compound 6) was added to a **liposome** suspension. The spectrum obtained for PKH26, a known cyanine dye, is also shown for comparison.

Dwg.2/4

L46 ANSWER 3 OF 32 MEDLINE DUPLICATE 1
2000031546 Document Number: 20031546. Cellular uptake of adamantyl conjugated peptide nucleic acids. Ljungstrom T; Knudsen H; Nielsen P E. (Center for Biomolecular Recognition, Department of Medical Biochemistry & Genetics, Biochemistry Laboratory B, The Panum Institute, Blegdamsvej 3c, 2200 Copenhagen N, Denmark.) BIOCONJUGATE CHEMISTRY, (1999 Nov-Dec) 10 (6) 965-72. Journal code: A1T. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB Peptide nucleic acids (PNA) (15-mers) conjugated to adamantyl acetic acid and **labeled** with fluorescein have been prepared, and their (**liposome** mediated) uptake in human cells in culture (HeLa, IMR-90 and MDA-MB-453) has been studied by confocal **fluorescence microscopy**. It is found that adamantyl-PNAs show greatly improved (endosomal) cellular uptake, but that this uptake is dependent on the cell line. Cellular uptake of such **lipophilic** PNAs is further mediated by cationic **liposomes**, and in some cases, the intracellular localization is diffuse cytoplasmic or nuclear, again cell-type dependent. The results show that this simple PNA modification can indeed greatly improve cellular uptake, but the effect appears strongly cell-type as well as PNA-sequence dependent.

L46 ANSWER 4 OF 32 MEDLINE DUPLICATE 2
2000046323 Document Number: 20046323. Vasodilating effect and tissue accumulation of prostaglandin E1 incorporated in lipid microspheres on the rat ductus arteriosus. Chino Y; Minagawa T; Kohno Y; Fukushima K; Momma K. (Research Center, Taisho Pharmaceutical Co., Ltd., Ohmiya, Saitama, Japan.) JAPANESE JOURNAL OF PHARMACOLOGY, (1999 Sep) 81 (1) 107-14. Journal code: KO7. ISSN: 0021-5198. Pub. country: Japan. Language: English.

AB Prostaglandin E1 incorporated in lipid microspheres (lipo PGE1) was administered to the umbilical vein of neonatal rats. **Morphological** measurement and quantitative autoradioluminography assessed the relationship between the vasodilating effect and tissue accumulation of lipo PGE1 in the ductus arteriosus. In the **morphological** measurement under **microscopy**, the inner diameter ratio of the ductus arteriosus to the main pulmonary artery after infusion of 3H-**labeled** lipo PGE1 (3H-lipo PGE1) continued to remain significantly higher than that of free 3H-PGE1. Autoradioluminography of the frozen frontal section of neonates after intravenous infusion of 3H-lipo PGE1 for 2 h revealed that the ductus levels of radioactivity were higher than those of free 3H-PGE1 in saline solution, although the blood levels were almost equal. Localization of lipo PGE1 **labeled** with a **lipophilic fluorescent** probe, 1,1'-dioctadecyl-3,3,3',3-tetramethyl-indocarbocyanine perchlorate (diI), in the endothelial cells of the ductus arteriosus was confirmed by confocal laser scanning **microscopy**. These findings suggest that the incorporation of lipid microspheres by the endothelial cells is one of the mechanisms that enables lipo PGE1 to accumulate to higher levels in the ductus tissue and to act more efficiently than free PGE1 in neonatal rats.

L46 ANSWER 5 OF 32 MEDLINE DUPLICATE 3

1999019840 Document Number: 99019840. Application of vesicles to rat skin in vivo: a confocal laser scanning **microscopy** study. van Kuijk-Meuwissen M E; Mougin L; Junginger H E; Bouwstra J A. (Division of Pharmaceutical Technology, Leiden/Amsterdam Center of Drug Research (LACDR), Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands.) J Controlled Release, (1998 Dec 4) 56 (1-3) 189-96. Journal code: C46. ISSN: 0168-3659. Pub. country: Netherlands. Language: English.

AB A major problem in (trans)dermal drug delivery is the low penetration rate of most substances through the barrier of the skin, the stratum corneum. One of the methods to increase the penetration rate across the skin is encapsulation of a (model) drug in lipid vesicles. In this study **fluorescently labelled liposomes** were applied on rat skin, in vivo. Bilayer **labelled** gel-state and liquid-state **liposomes** (conventional or with flexible bilayers) were non-occlusively applied on the dorsal area in the neck of the rat for 1, 3 or 6 h. Micelles were used as a control formulation. The penetration pathway and penetration depth of the **lipophilic fluorescent label** into the skin was visualised by confocal laser scanning **microscopy** (CLSM). During the first 3 h of application almost no differences in penetration depth were observed, when the **label** was applied in the various formulations. After 6 h application, it was clear that the **label** applied in micelles and gel-state **liposomes** did not penetrate as deep into the skin as the **label** applied in liquid-state vesicles. Among the liquid-state vesicles, the suspension with the most flexible bilayers showed the highest **fluorescence** intensity in the viable epidermis and dermis, 6 h post-application. Thus the vesicular form and the thermodynamic state of the bilayer and to a smaller extent the flexibility of the bilayer influence the penetration depth of the **label** into the skin at longer application periods. These results are in good agreement with CLSM results obtained from in vitro experiments with human skin.

L46 ANSWER 6 OF 32 MEDLINE DUPLICATE 4

1998234283 Document Number: 98234283. Interactions between **liposomes** and human skin in vitro, a confocal laser scanning **microscopy** study. van Kuijk-Meuwissen M E; Junginger H E; Bouwstra J A. (Division of Pharmaceutical Technology, Leiden/Amsterdam Center of Drug Research, Leiden University, P.O. Box 9502, Leiden 2300RA,The Netherlands.. meuwisse@chem.leidenuniv.nl) . BIOCHIMICA ET BIOPHYSICA ACTA, (1998 Apr 22) 1371 (1) 31-9. Journal code: A0W. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB One major problem in (trans)dermal drug delivery is the low penetration rate of drugs through the barrier of the skin. Encapsulation of a drug in lipid vesicles is one strategy to increase the penetration rate of a drug across the skin. In this study, the interactions between **fluorescent-labelled liposomes** and skin are visualized by confocal laser scanning **microscopy** (CLSM). Bilayer **labelled** gel-state and liquid-state **liposomes** (conventional or with flexible bilayers) were non-occlusively applied on human skin in vitro. The penetration pathway and penetration depth of the

lipophilic fluorescent label into the skin were visualized. From the CLSM images, it was clear that the **label** applied in micelles and gel-state **liposomes** did not penetrate as deep into the skin as the **label** applied in liquid-state vesicles. Among the liquid-state vesicles, the suspensions with the flexible bilayers showed the highest **fluorescence** intensity in the dermis. Thus, the thermodynamic state of the bilayer and, to a smaller extent, the flexibility of the bilayer influence, strongly the penetration depth of the **label** into the skin. The **label** applied non-occlusively in flexible **liposomes** penetrated deeper into the skin than after occlusive application. Copyright 1998 Elsevier Science B.V.

L46 ANSWER 7 OF 32 MEDLINE

97405905 Document Number: 97405905. Trompl, a putative rare outer membrane protein, is anchored by an uncleaved signal sequence to the Treponema pallidum cytoplasmic membrane. Akins D R; Robinson E; Shevchenko D; Elkins C; Cox D L; Radolf J D. (Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas 75235, USA.) JOURNAL OF BACTERIOLOGY, (1997 Aug) 179 (16) 5076-86. Journal code: HH3. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB Treponema pallidum rare outer membrane protein 1 (Trompl) has extensive sequence homology with substrate-binding proteins of ATP-binding cassette transporters. Because such proteins typically are periplasmic or cytoplasmic membrane associated, experiments were conducted to clarify Trompl's physicochemical properties and cellular location in T. pallidum. Comparison of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis mobilities of (i) native Trompl and Trompl synthesized by coupled in vitro transcription-translation and (ii) native Trompl and recombinant Trompl lacking the N-terminal signal sequence revealed that the native protein is not processed. Other studies demonstrated that recombinant Trompl lacks three basic porin-like properties: (i) the ability to form aqueous channels in **liposomes** which permit the influx of small hydrophilic solutes, (ii) an extensive beta-sheet secondary structure, and (iii) amphiphilicity. Subsurface localization of native Trompl was demonstrated by immunofluorescence analysis of treponemes encapsulated in gel microdroplets, while opsonization assays failed to **detect** surface-exposed Trompl. Incubation of motile treponemes with 3-(trifluoromethyl)-3-(m-[125I]iodophenyl)-diazarine, a photoactivatable, **lipophilic** probe, also did not result in the **detection** of Trompl within the outer membranes of intact treponemes but, instead, resulted in the **labeling** of a basic 30.5-kDa presumptive outer membrane protein. Finally, analysis of fractionated treponemes revealed that native Trompl is associated predominantly with cell cylinders. These findings comprise a body of evidence that Trompl actually is anchored by an uncleaved signal sequence to the periplasmic face of the T. pallidum cytoplasmic membrane, where it likely subserves a transport-related function.

L46 ANSWER 8 OF 32 MEDLINE

97079513 Document Number: 97079513. Impregnation of collagen corneal shields with **liposomes**: uptake and release of hydrophilic and

lipophilic marker substances. Grammer J B; Kortum F A; Wolburg H; Ludtke R; Schmidt K H; Thiel H J; Pleyer U. (Department of Surgery, University of Tubingen, Germany.) CURRENT EYE RESEARCH, (1996 Aug) 15 (8)
815-23. Journal code: DUB. ISSN: 0271-3683. Pub. country: ENGLAND: United
Kingdom. Language: English.

AB PURPOSE: **Liposomes** and collagen corneal shields (CCS) have been used as ophthalmic drug delivery devices. With regard to a possibly combined application, we studied the effects of surface charge and bilayer
fluidity of **liposomes** on their uptake and release by CCS.
METHODS: 12-hours-CCS were soaked in large unilamellar **liposomes**, which had been **labelled** with 4,5-carboxyfluorescein (CF) and N-(lissamine rhodamine B sulfonyl)-diacyl-phosphatidylethanolamine (PE-RhB) in the aqueous space and in the **liposome** bilayer, respectively. Released fluorophores were determined fluorometrically in the elution buffer at intervals from 1 to 240 min after immersion.
RESULTS: The CF concentration in the CCS soaked in a CF solution was two to seven times higher than immersion in the **liposome** suspensions. Among those, the negatively charged, cholesterol-containing preparation led to the highest CF concentration in the CCS. The PE-RhB concentration was highest after soaking the CCS in neutral, cholesterol-free **liposomes**. All types of **liposomes** were found inside the CCS by freeze fracture electron **microscopy**. The release kinetics data indicate a first order release. More than 90% of CF was released by the CCS within the first 30 min. This was equal after soaking the CCS in the CF solution or in **liposomes**. With DOPC-**liposomes**, the maximal release was already attained after 10 min. In general, the differences in the release kinetics of both hydrophilic and **lipophilic** markers, obtained by the various **liposome** types were small. CONCLUSIONS: Our results indicate that surface charge and bilayer fluidity are of minor importance for the interaction with collagen corneal shields. However, since the release kinetics of a **liposome**-encapsulated hydrophilic or **lipophilic** substance are similar to the release of a non-encapsulated drug, the combination of **liposomes** with collagen shields may be useful mainly with respect to the encapsulation of
drugs which do not penetrate the ocular surface as well as to prolong corneal contact time of the **liposomes**.

L46 ANSWER 9 OF 32 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
96030678 EMBASE Document No.: 1996030678. **Fluorescence** generalized polarization of **cell membranes**: A two-photon scanning **microscopy** approach. Yu W.; So P.T.C.; French T.; Gratton E.. Laboratory for Fluorescence Dynamics, Department of Physics, University of
Illinois, 1110 W. Green,Urbana, IL 61801, United States. Biophysical Journal 70/2 I (626-636) 1996.
ISSN: 0006-3495. CODEN: BIOJAU. Pub. Country: United States. Language: English. Summary Language: English.

AB We use the **lipophilic fluorescence** probe Laurdan to study **cell membranes**. The generalized polarization (GP) of Laurdan-**labeled** cells contains useful information about membrane fluidity and polarity. A high GP is usually associated with low

fluidity, low polarity, or high cholesterol content of the membranes, and a low GP is the opposite. We have combined the GP method and two-photon **fluorescence microscopy** to provide an alternative approach to study **cell membranes**. Using two-photon excitation in a conventional **microscope** offers great advantages for studying biological samples. These advantages include efficient background rejection, low photodamage, and improved depth discrimination. We performed GP measurements on mouse fibroblast cells and observed that both intensity and GP images are not spatially uniform. We tested for possible GP artifacts arising from cellular autofluorescence and lifetime quenching, using a procedure for background **fluorescence** subtraction and by direct lifetime measurements in the **microscope**. GP measured in a single cell displays a broad distribution, and the GP of 40 different cells grown on the same cover glass is also statistically distributed. The correlations between intensity and GP images were analyzed, and no monotonic dependence between the two was found. By digitally separating high and low GP values, we found that high GP values often associate with the regions of the plasma membrane and low GP values link with the nuclear membranes. Our results also show local GP variations within the plasma and nuclear membranes.

L46 ANSWER 10 OF 32 CAPLUS COPYRIGHT 2000 ACS

1996:418671 Document No. 125:109030 Targeting of the tumor microcirculation with a new photosensitizer. Abels, C.; Dellian, M.; Szeimies, R. -M.; Steinbach, P.; Richert, C.; Goetz, A. E. (Institutes Surgical Research, Ludwig-Maximilians-University, Munich, 81366, Germany). Proc. SPIE-Int. Soc. Opt. Eng., 2625(Photochemistry: Photodynamic Therapy and Other Modalities), 164-169 (English) 1996. CODEN: PSISDG. ISSN: 0277-786X.

AB Tumors are characterized by an insufficient neoangiogenesis. Therefore targeting of the fragile tumor microcirculation by photodynamic therapy (PDT) may induce easily tumor ischemia leading to tumor necrosis. 9-Acetoxy-2,7,12,17-tetrakis-(.beta.-methoxyethyl)-porphycene (ATMPn) is a CP, **lipophilic** substance and revealed superior photodynamic characteristics in vitro as compared to Photofrin. In this study pharmacokinetics, photodynamic effects and localization of ATMPn incorporated in small unilamellar **liposomes** in tumor and surrounding normal tissue were evaluated. Amelanotic melanomas (A-Mel-3) were implanted in dorsal skin fold chambers fitted to Syrian Golden hamsters (70-80 g b.w.). **Fluorescence** kinetics of ATMPn administered i.v. (1.4 .mu.mol/kg b.w.; n=8) was monitored by intravital **microscopy**. Quant. measurements of **fluorescence** intensity were carried out by digital image anal. For tumor growth studies 1.4 .mu.mol/kg was injected 24 h (n=3), 3 h (n=3), 1 min (n=6) and 2.8 .mu.mol/kg 1 min (n=6) before PDT (630 nm, 100 mW/cm2, 100 J/cm2). Tumor growth was measured over 28 d. Solid tumors (n=3) were excised 1 min after injection of ATMPn (1.4 .mu.mol/kg) and cryostat sections (10 .mu.m) were analyzed by confocal laser scanning **microscopy** (CSLM) to det. tissue localization of dye. Maximal **fluorescence** (mean.+-.S.E.) arose in tumor (94.+-.7%) and surrounding host tissue (67.+-.5%) 30 s post injection followed by a rapid decrease. Hardly any **fluorescence** was **detectable** after 12 h. Only PDT 1 min after injection of ATMPn was effective yielding 1/6 complete remission (1.4 .mu.mol/kg) and 3/6 complete remissions (2.8 mmol/kg), resp. At that

time dye is primarily localized in vessels and vessel walls as shown by CLSM. ATMPn in **liposomes** reveals very rapid kinetics thus suitable for intraoperative PDT. Moreover, PDT (2.8 .mu.mol/kg) at time, when dye is localized in tumor microcirculation, exhibits best tumor killing effects.

L46 ANSWER 11 OF 32 MEDLINE DUPLICATE 5
97155550 Document Number: 97155550. Dynamic **fluorescence** changes during photodynamic therapy in vivo and in vitro of hydrophilic Al(III) phthalocyanine tetrasulphonate and **lipophilic** Zn(II) phthalocyanine administered in **liposomes**. Ruck A; Beck G; Bachor R; Akgun N; Gschwend M H; Steiner R. (Institut fur Lasertechnologien in der Medizin und Messtechnik, Ulm, Germany.) JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY. B, BIOLOGY, (1996 Nov) 36 (2) 127-33. Journal code: JLI. ISSN: 1011-1344. Pub. country: Switzerland. Language: English.
AB The **fluorescence emission** of hydrophilic tetrasulphonated aluminium phthalocyanine (AlPcS4) and hydrophobic zinc phthalocyanine (ZnPc), bound to the membrane of **liposomes**, was investigated in vivo in an appropriate tumour model of the rat bladder and in RR 1022 epithelial cells of the rat. The sensitizers were administered systemically to the rats and photodynamic therapy (PDT) was performed 24 h later. During PDT treatment, the **fluorescence** was measured every 30 s. The **fluorescence** was **excited** with 633 nm light from an HeNe laser and the **fluorescence** spectra were **detected** with an optical multichannel analyser system. PDT was performed for both sensitizers using 672 nm light from an Ar+ dye laser. The **fluorescence** changes during PDT were significantly different for the two phthalocyanines. For AlPcS4, an initial **fluorescence** intensity increase, followed by subsequent photobleaching, was observed. In contrast, ZnPc **fluorescence** showed an exponential decrease and no increase at the start of treatment. Tumour necrosis 24 h after PDT was significant only for ZnPc. RR 1022 cells incubated for 24 h with AlPcS4 revealed a granular **fluorescence** pattern, whereas ZnPc was localized diffusely in the cytoplasm of the cells. In agreement with the in vivo measurements, subcellular relocalization and a **fluorescence** intensity increase were **detected** exclusively in the case of AlPcS4. **Morphological** changes at this time were significant only for ZnPc. The subcellular localization and **fluorescence** kinetics were obtained using a confocal laser scanning **microscope**.

L46 ANSWER 12 OF 32 CAPLUS COPYRIGHT 2000 ACS
1996:721071 Document No. 126:11513 Passive targeting to solid tumor by long-circulating **liposomes**. Unezaki, Sakae; Hosoda, Jun-ichi; Maruyama, Kazuo; Iwatsuru, Motoharu (Department Pharmacy, Tokyo Medical College Hospital, Tokyo, 160, Japan). Organ Biol., 3(3), 61-68 (English) 1996. CODEN: ORBIF3. ISSN: 1340-5152. Publisher: Nippon Zoki Hozon Seibutsu Igakkai.
AB Enhanced delivery of doxorubicin (DXR) to colon 26 in mice was studied by the long-circulating **liposomes** composed of distearoylphosphatidylcholine/cholesterol (1/1 molar ratio) and 6 mol% amphipathic poly(ethylene glycol) (PEG), and the extravasation of PEG-coated **liposomes** (PEG-Lipo) **labeled** with DiI (

lipophilic dye) in mouse neuroblastoma C-1300 solid tumor was visualized by in vivo **fluorescence microscopy**. DXR-PEG-Lipo showed high blood levels and the greatest tumor accumulation compared with free DXR and DXR-Lipo. Moreover, administration of DXR-PEG-Lipo resulted in effective retardation of tumor growth and prolongation fo survival times. With time after injection of DiI-PEG-Lipo, the tumor interstitial **fluorescence** intensity increased. Most **fluorescent** spots were located outside and around the vessel wall, indicating extravasation of intact **liposomes**. The authors also obtained the same **fluorescence** localization pattern with DXR released from extravasate DXR-PEG-Lipo after injection. The present study provides a possible mechanistic basis for the increased tumor accumulation of long-circulating **liposomes**. Thus long-circulating **liposomes** should be useful carriers of chemotherapeutic agents for the treatment of solid tumor.

L46 ANSWER 13 OF 32 CAPLUS COPYRIGHT 2000 ACS
1996:99396 Document No. 124:140411 Assay for determining biochemical changes at phospholipid bilayer membrane surfaces. O'Shea, Paul Stuart (British Technology Group Ltd., UK). Brit. UK Pat. Appl. GB 2288019 A1 19951004, 30 pp. (English). CODEN: BAXXDU. APPLICATION: GB 1995-6592 19950330. PRIORITY: GB 1994-6464 19940331.

AB A method of **detecting** or measuring cell receptors and other mols. near the surface of cells having a **lipid membrane**, esp. human lymphocytes and erythrocytes, is disclosed in which the cells are **labeled** in vitro with a mol. probe comprising in the mol. a **lipophilic** moiety so that the probe can insert into the **cell membrane** and a reporter moiety of the pH-indicating kind, esp. one which is **fluorescent**, which will stand above the cell surface, causing a ligand to bind to the receptor or other cell surface mol. and **detecting** or measuring the resultant change in the elec. potential of the probe, esp. a change in **fluorescence** intensity. Fluorescein phosphatidylethanolamine is a preferred probe. The invention is also applicable to **liposomes** and lipid bilayers.

L46 ANSWER 14 OF 32 MEDLINE DUPLICATE 6
95403446 Document Number: 95403446. Characterization of calcium translocation across the plasma membrane of primary osteoblasts using a **lipophilic** calcium-sensitive **fluorescent** dye, calcium green C18. Lloyd Q P; Kuhn M A; Gay C V. (Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park 16802, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Sep 22) 270 (38) 22445-51. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The synthesis of Calcium Green C18, a **lipophilic** **fluorescent** calcium-sensitive dye, and its use as a monitor of Ca2+ efflux from cells is described. This indicator consists of a Calcium Green-1 molecule conjugated to a **lipophilic** 18-carbon alkyl chain which will intercalate into **cell membranes**. The Kd of the indicator for Ca2+ in aqueous solution (pH 7.2, 22 degrees C, ionic strength 0.1 M) is 0.23 +/- 0.04 microM and in the presence of

liposomes is 0.062 +/- 0.007 microM. Due to its high negativity, the calcium chelating fluorophore faces the cell exterior, when loaded under a defined set of conditions. The dye was found largely on the surface of the cells when loaded at a concentration of 5 microM for 10 min at 37 degrees C. Five minutes after introduction of EGTA, 83-95% **fluorescence** disappeared, indicating that most of the fluorophore was on the cell surface. Photobleaching was minimal (3-13%). A confocal laser scanning **microscope** was used to **detect** and quantify **fluorescence**. Internalized dye was apparent in cells loaded for longer times (30-60 min) and in membrane-impaired cells, as shown by uptake of propidium iodide. Under defined confocal laser scanning **microscope** settings, a transient **fluorescence** at the periphery of approximately 30% of the cells was observed following 10(-8) M parathyroid hormone treatment, indicating the presence of outwardly directed calcium transport across the plasma membrane. Calcium efflux usually lasted 7-10 min, peaking at around 2-3 min. Changes in cell shape were also observed. Calcium efflux was shown to be sensitive to (a) 10 microM quercetin and 10 microM vanadate, partially specific inhibitors of plasma membrane Ca(2+)-ATPase, to (b) 0.1 mM trifluoperazine, an agent which renders calmodulin ineffective, and to (c) 10 mM neomycin sulfate, which blocks release of Ca2+ from intracellular stores. Thapsigargin (5 microM), an inhibitor of Ca(2+)-ATPase of the endoplasmic reticulum, prolonged **fluorescence**. These observations indicate that cell surface **fluorescence** was due to the capture of Ca2+ by Calcium Green C18 after Ca2+ had been translocated across osteoblast plasma membranes. Involvement of the plasma membrane Ca(2+)-ATPase, known to be present in osteoblasts in substantial amounts, is implicated.

L46 ANSWER 15 OF 32 MEDLINE
96150890 Document Number: 96150890. Internalization of microbubbles by tumor cells in vivo and in vitro. Barbarese E; Ho S Y; D'Arrigo J S; Simon R H. (Dept. of Neurology, University of Connecticut Health Center, USA.) JOURNAL OF NEURO-ONCOLOGY, (1995 Oct) 26 (1) 25-34. Journal code: JCP. ISSN: 0167-594X. Pub. country: Netherlands. Language: English.

AB Lipid-coated microbubbles (LCM) administered intravenously (i.v.) to rats bearing brain tumor, specifically enhance tumor visualization by ultrasound [1]. In order to understand the basis for this observation, we have examined the interactions of LCM with glioblastoma (C6) and gliosarcoma (9L) tumor cells in vivo and in vitro. LCM and LCM **labeled** with the **fluorescent lipophilic** dye 3,3'-dioctadecyloxacarbocyanine perchlorate (diO) were administered to rats bearing brain tumor. LCM and diO-**labeled** LCM were found principally at the tumor site with no evidence of **label** in the surrounding normal brain tissue. Analysis of the tumor by confocal laser scanning **microscopy** revealed that **labeled** LCM were inside the tumor cells. Similar analysis of LCM interactions with C6 and 9L cells in culture showed that LCM first adsorb at the surface of the cells, and with time became localized inside the cells. Binding and internalization proceeded faster at 37 degrees C than at room temperature (RT). Staining of live cells with N-(3-((2,4-dinitrophenyl)amino)propyl)-N-(3-aminopropyl) methylamine dihydrochloride (DAMP), a dye that recognizes acidic compartments, showed that the majority of internalized LCM was

associated with compartments containing DAMP. If the same uptake mechanism
were operative in vivo, it would indicate that a portion of LCM bypasses the reticuloendothelial system and become endocytosed directly by tumor cells.

L46 ANSWER 16 OF 32 MEDLINE DUPLICATE 7
95015966 Document Number: 95015966. Localization of **liposomes** containing a DNA repair enzyme in murine skin. Yarosh D; Bucana C; Cox P; Alas L; Kibitel J; Kripke M. (Applied Genetics Inc., Freeport, New York 11520.) JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1994 Oct) 103 (4) 461-8. Journal code: IHZ. ISSN: 0022-202X. Pub. country: United States. Language:
English.
AB T4N5 **liposomes**, which contain the DNA repair enzyme T4 endonuclease V, were applied to mouse skin in vivo and added to cultured murine keratinocytes in vitro. The fate of the **liposome** membrane was followed using a **fluorescent**, **lipophilic** dye, and the fate of the enzyme was traced by immunogold **labeling**, followed by brightfield, **fluorescence**, or transmission electron **microscopy**. In vivo, T4N5 **liposomes** penetrated the stratum corneum, localized in epidermis and appendages of the skin, and were found inside basal keratinocytes. The enzyme was found inside keratinocytes treated in vitro and in the epidermis, hair follicles, and sebaceous glands of topically treated skin. Ultrastructural studies demonstrated the presence of **liposomes** in the cytoplasm of cells in the epidermis often concentrated in a perinuclear location. The enzyme was present in both nucleus and cytoplasm of keratinocytes and Langerhans cells. **Liposomes** were found in cells of the lymph nodes draining the site of contact sensitization, in association with topically applied antigen. The results demonstrate that **liposomes** can deliver encapsulated proteins into cells of the skin in vivo and provide insight into how **liposome**-enhanced DNA repair reduces UV-induced skin cancer and systemic immunosuppression in mice.

L46 ANSWER 17 OF 32 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
93352574 EMBASE Document No.: 1993352574. Permeability enhancement in Caco-2 cell monolayers by sodium salicylate and sodium taurodihydrofusidate: Assessment of effect-reversibility and imaging of transepithelial transport routes by confocal laser scanning **microscopy**. Hurni M.A.; Noach A.B.J.; Blom-Roosemalen M.C.M.; De Boer A.G.; Nagelkerke J.F.;
Breimer D.D.. Division of Pharmacology, Leiden/Amsterdam Drug Research Ctr., Bio-Pharmaceutical Sciences Center, P.O. Box 9503,NL-2300 RA Leiden,
Netherlands. Journal of Pharmacology and Experimental Therapeutics 267/2 (942-950) 1993.
ISSN: 0022-3565. CODEN: JPETAB. Pub. Country: United States. Language: English. Summary Language: English.
AB The effects of sodium salicylate and sodium tauro-24, 25-dihydrofusidate (STDHF) on the aqueous permeability of confluent monolayers of Caco-2 cells were studied. Measurements of transepithelial electrical resistance (TEER) showed a concentration-dependent effect of both compounds after apical incubation for 1 hr. Reductions in TEER resulting from EC50 concentrations (2.8 mM for STDHF; 173 mM for salicylate) were reversible within 5.75 hr. The transepithelial fluxes of two hydrophilic model

compounds, sodium fluorescein F (molecular weight 376) and a fluorescein isothiocyanate-**labeled** dextran (mean molecular weight 4000) was significantly increased by STDHF (2.8 mM). Sodium salicylate (173 mM) only enhanced the transport of sodium fluorescein significantly. At the EC50 concentrations, confocal laser scanning **microscopy** (CLSM) visualized both **fluorescent** tracers mainly in the paracellular route. With higher enhancer concentrations (373 mM sodium salicylate and 8 mM STDHF), both transport markers appeared intracellularly as a result of cell death. STDHF rapidly extracted an exogenous **lipophilic** membrane probe, 5-(N-hexadecanoyl)aminofluorescein (HEDAF), from the apical part of Caco-2 plasma membranes, indicating qualitatively that STDHF interacts with the lipid portion of **cell membranes** . These results suggest that both sodium salicylate and STDHF can be used to reversibly increase paracellular permeability of Caco-2 cell monolayers, whereby STDHF appears to be advantageous compared to sodium salicylate. By adapting the Costar cell culture system to CLSM, we have shown that this technique is suitable to study membrane interactions qualitatively and for visualizing transport routes of hydrophilic tracers through nonfixed, **filter**-grown monolayers.

L46 ANSWER 18 OF 32 MEDLINE DUPLICATE 8

94163749 Document Number: 94163749. Sphingosine-mediated membrane association of DNA and its reversal by phosphatidic acid. Kinnunen P K; Rytomaa M; Koiv A; Lehtonen J; Mustonen P; Aro A. (Department of Medical Chemistry, University of Helsinki, Finland..) CHEMISTRY AND PHYSICS OF LIPIDS, (1993 Nov) 66 (1-2) 75-85. Journal code: CZW. ISSN: 0009-3084. Pub. country: Ireland. Language: English.

AB Resonance energy transfer was measured between egg phosphatidylcholine **liposomes** containing the intramolecular excimer forming pyrene-**labelled** phospholipid analogue 1,2-bis[pyren-1-(-yl)]decanoyl-sn-glycero-3-phosphocholine (bisPDPC) as a donor and DNA-bound adriamycin as an acceptor. Membrane association of DNA turned out to be critically dependent on the presence of sphingosine in the **liposomes**. Identical result was obtained by measuring the extent of quenching of the **fluorescent** DNA-bound dye Hoechst 33258 due to energy transfer to the **lipophilic** stain Nile Red incorporated in egg phosphatidylcholine **liposomes** containing varying amounts of sphingosine. The attachment of DNA to sphingosine-containing membranes could be reversed by the further inclusion of the negatively charged phosphatidic acid up to approximately 1:2 PA/sphingosine molar ratio in the **liposomes**, thus suggesting the involvement of electrostatic interactions. Differential scanning calorimetry measurements confirmed a lack of association between DNA and dimyristoylphosphatidylcholine **liposomes**. Instead drastic changes were produced by DNA in the heat capacity scans measured for **liposomes** also incorporating sphingosine. **Fluorescence microscopy** revealed an extensive aggregation of sphingosine containing pyrene-phosphatidylcholine-**labelled** egg phosphatidylcholine **liposomes** in the presence of DNA. Together with other available data on the effects of sphingosine, the present findings suggest that sphingosine could directly alter the chromatin structure. Accordingly, such alterations may contribute to the control of replication and gene expression.

L46 ANSWER 19 OF 32 MEDLINE DUPLICATE 9
94011007 Document Number: 94011007. In vivo distribution of particulate antigens and **liposomes** in murine spleen. A possible role in the humoral immune response. Buiting A M; de Rover Z; Claassen E; van Rooijen N. (Department of Cell Biology, Medical Faculty, Vrije University, Amsterdam, The Netherlands..) IMMUNOBIOLOGY, (1993 Jun) 188 (1-2) 13-22. Journal code: GH3. ISSN: 0171-2985. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Several particulate antigens and **liposomes** were intravenously injected in mice in order to study their localization patterns in spleen and liver. **Liposomes** have been proposed as promising carriers for haptens and antigens. It was studied whether the phospholipid composition, cholesterol content and charge of the **liposomes** played a role in their distribution within the spleen. Different thymus-independent type 1 and type 2 and thymus-dependent particulate antigens as well as **liposomes** were **labeled** with the **lipophilic** fluorochrome Di-I. After **labeling** they were intravenously injected and spleens and livers were removed at different time intervals and prepared for light- and **fluorescence-microscopy**. We have observed that all particulate antigens and **liposomes** administered to the mice localized according to the same distribution pattern in the spleen. After 2 and 4 h particles were located in macrophages of the marginal zone and after 24 h white pulp macrophages had also ingested particulate antigens and **liposomes**. So we conclude that the distribution of the particulate antigens and **liposomes** in the spleen is independent of the immunological nature of the particles. Results are discussed with respect to the question whether or not the distribution of particulate antigens and **liposome** associated antigens or haptens, may be a crucial factor in determining the type of immune response to be elicited.

L46 ANSWER 20 OF 32 MEDLINE DUPLICATE 10
92193640 Document Number: 92193640. Post-formation **fluorescent labelling** of liposomal membranes. In vivo **detection**, localisation and kinetics. Claassen E. (Department of Immunology and Medical Microbiology, TNO Medical Biological Laboratory, Rijswijk, Netherlands.) JOURNAL OF IMMUNOLOGICAL METHODS, (1992 Mar 4) 147 (2) 231-40. Journal code: IFE. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB A fast and simple method for the in vivo/in situ **detection** of **liposomes** is described. Utilizing **lipophilic** carbocyanine dyes, DiI and DiO, yellow (or red) and green **fluorescent liposomes** can be visualised with routinely available **filters**. The main advantages of the method are (i) the vesicles can be **labelled** after they are formed and (ii) the **label** does not interfere with proteins on the surface of the **liposomes**. **Labelled liposomes** were found in macrophages of spleen and liver (of mice) within 30 min of intravenous administration. In the spleen, **labelled liposomes** localised preferentially in the marginal zone macrophages, as confirmed by double staining with FITC-Ficoll. These data correlate well with the fact that empty or haptenated **liposomes** are thymus-independent antigens, and that other thymus-independent antigens are also specifically

taken up by marginal zone macrophages. The immunological role of these macrophages in the processing and presentation of antigen-bearing **liposomes** can now be studied in more detail. Administration of high doses (1-3 mg lipid) of **labelled liposomes** showed that uptake occurred preferentially, but not exclusively, by marginal zone
macrophages. After the marginal zone macrophages had been 'saturated', the
red pulp macrophages took up the **liposomes**. DiI and DiO have also been successfully used for **labelling** lymphocytes and bacteria for in vivo homing studies. The fact that **liposomes** can be **labelled** after they have been formed is an advantage for retrospective (i.e. **liposomes** already in use/storage) studies in e.g. targeting of drugs by **liposomes**.

L46 ANSWER 21 OF 32 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
92015705 EMBASE Document No.: 1992015705. How deep do intact **liposomes** penetrate into human skin?. Lasch J.; Laub R.; Wohlrab W.. Institute of Biochemistry, Medical Faculty, Martin-Luther-University, Hollystrasse 1,D 0-4020 Halle/Saale, Germany. Journal of Controlled Release 18/1 (55-58) 1992.
ISSN: 0168-3659. CODEN: JCREEC. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB Attempts were made to visualise intact **liposomes** in the human skin by fluoromicrography. To this end the aqueous compartment of the **liposomes** as well as the lipid bilayer were followed by **labelling** with hydrophilic and **lipophilic** fluorophores, respectively. Micrographs at various times after external application suggested that intact **liposomes** do not penetrate deeper than the horny layer.

L46 ANSWER 22 OF 32 MEDLINE DUPLICATE 11
91268091 Document Number: 91268091. Distance between skeletal protein 4.1 and the erythrocyte membrane bilayer measured by resonance energy transfer. Shahrokh Z; Verkman A S; Shohet S B. (Department of Laboratory Medicine, University of California, San Francisco 94143..) JOURNAL OF BIOLOGICAL CHEMISTRY, (1991 Jun 25) 266 (18) 12082-9. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB To assess the molecular architecture of the human erythrocyte skeletal protein 4.1:bilayer interface, the distance between a donor sulfhydryl-specific **fluorescent** probe attached to a region near the glycophorin-binding domain of protein 4.1 and an acceptor **lipophilic** probe in the exposed leaflet of inside-out vesicles (IOVs) was measured by **fluorescence** resonance energy transfer. To prevent aggregation and loss of function, protein 4.1 was **labeled** in situ on the surface of IOVs, purified, and rebound onto fresh IOVs. The **labeled** protein 4.1 was similar to the native protein in its gel electrophoretic pattern and its binding affinity to stripped-IOVs (Kd 35 +/- 4 nM). Energy transfer was assessed using two donor-acceptor pairs, 5-[2-[(iodoacetyl)amino]ethyl] aminonaphthalene-1-sulfonic acid and 3,3'-ditetradecyloxacarbocyanine perchlorate, or 5-iodoacetamidofluorescein and tetramethylrhodamine phosphatidylethanolamine. Using both donor **fluorescence** intensity and lifetime quenching measurements, an average distance of 75 +/- 5 A between the probe on the protein and the surface of IOVs was found. In parallel **fluorescence** resonance energy transfer

studies with protein 4.1 and **liposomes** with a phospholipid composition similar to the inner leaflet of the red **cell membrane**, a closer distance was found (49 +/- 5 A). Two control experiments validated energy transfer: (a) the spectrum of a mixture of IOVs separately **labeled** with donor and acceptor was different from the spectrum of the doubly **labeled** IOVs at identical donor and acceptor concentrations; and (b) no energy transfer was observed following detergent disruption of the geometric relationship between donor and acceptor. Taken together, these observations suggest that membrane-bound protein 4.1 is elongated and that the **labeled** site is located at a position deep in the 30-kDa N-terminal glycophorin-binding domain of the protein. The data are also consistent with the view that the cytoplasmic tail of glycophorin is interposed between protein 4.1 and the lipids. These experiments represent the first measurement of a distance between a skeletal protein and the lipid bilayer.

L46 ANSWER 23 OF 32 MEDLINE DUPLICATE 12

91341452 Document Number: 91341452. Fusion of influenza virions with a planar **lipid membrane detected** by video **fluorescence microscopy**. Niles W D; Cohen F S. (Department of Physiology, Rush Medical College, Chicago, IL 60612.) JOURNAL OF GENERAL PHYSIOLOGY, (1991 Jun) 97 (6) 1101-19. Journal code: I8N. ISSN: 0022-1295. Pub. country: United States. Language: English.

AB The fusion of individual influenza virions with a planar phospholipid membrane was **detected** by **fluorescence** video **microscopy**. Virion envelopes were loaded with the **lipophilic fluorescent** marker octadecylrhodamine B (R18) to a density at which the **fluorescence** of the probe was self-quenched. **Labeled** virions were ejected toward the planar membrane from a micropipette in a custom-built video **fluorescence microscope**. Once a virion fused with the planar membrane, the marker was free to diffuse, and its **fluorescence** became dequenched, producing a flash of light. This flash was **detected** as a transient spot of light which increased and then diminished in brightness. The diffusion constants calculated from the brightness profiles for the flashes are consistent with fusion of virus to the membrane with consequent free diffusion of probe within the planar membrane. Under conditions known to be fusigenic for influenza virus (low pH and 37 degrees C), flashes appeared at a high rate and the planar membrane quickly became **fluorescent**. To further establish that these flashes were due to fusion, we showed that red blood cells, which normally do not attach to planar membranes, were able to bind to membranes that had been exposed to virus under fusigenic conditions. The amount of binding correlated with the amount of flashing. This indicates that flashes signaled the reconstitution of the hemagglutinin glycoprotein (HA) of influenza virus, a well-known erythrocyte receptor, into the planar membrane, as would be expected in a fusion process. The flash rate on ganglioside-containing asolectin membranes increased as the pH was lowered. This is also consistent with the known fusion behavior of influenza virus with **cell membranes** and with phospholipid vesicles. We conclude that the flashes result from the fusion

of individual virions to the planar membrane.

L46 ANSWER 24 OF 32 CAPLUS COPYRIGHT 2000 ACS
1990:597851 Document No. 113:197851 Comparative studies of the preparation of immunoliposomes with the use of two bifunctional coupling agents and investigation of in vitro immunoliposome-target cell binding by cytofluorometry and electron **microscopy**. Schwendener, R. A.; Trueb, T.; Schott, H.; Langhals, H.; Barth, R. F.; Groscurth, P.; Hengartner, H. (Inst. Pathol., Univ. Hosp., Zurich, CH-8091, Switz.). Biochim. Biophys. Acta, 1026(1), 69-79 (English) 1990. CODEN: BBACAQ. ISSN: 0006-3002.
AB The two coupling agents, SPDP [N-succinimidyl-3-(2-pyridyldithio)propionate] and SATA [N-succinimidyl-S-acetylthioacetate), were compared in their efficiency and feasibility to couple monoclonal antibodies (Abs) via thioether linkage to **liposomes** functionalized by various **lipophilic** maleimide compds. like N-(3-maleimidopropionyl)-N2-palmitoyl-L-lysine Me ester (MP-PL), N-(3-maleimidopropionyl)phosphatidylethanolamide (MP-PE), N6-(6-maleimidocaproyl)-N2-palmitoyl-L-lysine Me ester (EMC-PL), and N-(6-maleimidocaproyl)phosphatidylethanolamine (EMC-PE). The compn. of the **liposomes** was soy phosphatidylcholine (SPC), cholesterol, maleimide compds. and .alpha.-tocopherol (1:0.2:0.02:0.01, mol parts), plus N4-oleylcytosine arabinoside (NOAC) as cytostatic prodrug (0.2 mol parts) and a new, **lipophilic** and highly **fluorescent** dye N,N'-bis(1-hexylheptyl)-2,3:9,10-perylenebis(dicarboximide)(BHPD, 0.006 mol parts). From the maleimide derivs. MP-PL was the most effective in terms of preservation of the coupling activity in dependence of **liposome** storage. The coupling of the monoclonal A B8-24.3 (mouse IgG2b, MHC class I, anti H-2kb) and IB16-6 (rat IgG2a, anti B16 mouse melanoma) to the drug-carrying **liposomes** was more effective and easier to accomplish with SATA as compared to SPDP. Coupling rates of 60-65% were obtained with SATA at molar ratios of 12 SATA:1 Ab:40 maleimide space groups on the surface of one **liposome**. The highest coupling rates with SPDP were obtained at the ratio of 24 SPDP:1 Ab:40 liposomal maleimide groups, with an Ab binding efficiency of only 20-25%. The optimal in vitro binding conditions to specific target cells (EL4 for B8-24.3-**liposomes** and B16-F10 for IB16-6-**liposomes**) were detd. by cytofluorometric measurement of the liposomal BHPD **fluorescence** with SATA linked Abs. Optimal immunoliposome binding to specific epitopes on the target cells was achieved with 1-2 Ab mols. coupled to one **liposome**, with immunoliposome concns. of 20-130 nM and with a small incubation vol. of 0.3-0.4 mL. The specificity of the binding of B8-24.3-**liposomes** to EL4 target cells was visualized by SEM. Antibody-mediated endocytic uptake of immunoliposomes could be demonstrated by transmission electron **microscopy**.

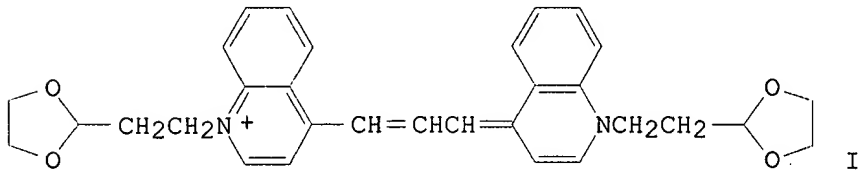
L46 ANSWER 25 OF 32 CAPLUS COPYRIGHT 2000 ACS
1988:34506 Document No. 108:34506 Membrane anchor conjugates with active agents, their preparation and uses. (Hoechst A.-G. , Fed. Rep. Ger.). Ger. Offen. DE 3546150 A1 19870122, 34 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1985-3546150 19851227. PRIORITY: DE 1985-3522512 19850624.
AB Active agents (antigens, antibiotics, hormones, enzymes, **labels**, etc.) are conjugated to compds. which can be inserted into **cell**

membranes. The conjugates are useful e.g. to promote cell fusion, to provide cells with **fluorescent** or spin **labels**, etc. The extracytoplasmic region of the EGF receptor encompassing residues 516-529 was constructed by the Merrifield resin method, coupled to fluorenylmethoxycarbonyl(tert-butyl)serine and S-[2,3-bis(palmitoyloxy)propyl]-N-palmitoylcysteinylserine(Pam3Cys-Ser) (the N-terminus of the outer membrane lipoprotein of Escherichia coli) as adjuvant, cleaved from the resin, and administered once i.p. to mice. A high titer of antibodies to the EGF receptor peptide was **detected** within 2 wk.

L46 ANSWER 26 OF 32 CAPLUS COPYRIGHT 2000 ACS

1987:191844 Document No. 106:191844 Photobleaching of a cyanine dye in solution and in membranes. Valdes-Aguilera, O.; Cincotta, L.; Foley, J.; Kochevar, I. E. (Dep. Dermatol. Harvard Med. Sch., Harvard Med. Sch., Boston, MA, 02114, USA). Photochem. Photobiol., 45(3), 337-44 (English) 1987. CODEN: PHCBAP. ISSN: 0031-8655.

GI



I

AB N,N'-Bis(2-ethyl-1,3-dioxolane)kryptocyanine (I), a **lipophilic** dye with a delocalized pos. charge, photosensitizes cells to visible irradn. In phosphate-buffered saline (PBS), I absorbs maximally at 700 nm (.epsilon. = 1.2 .times. 105 M-1 cm-1) and in MeOH the absorption max. is at 706 nm (.epsilon. = 2.3 .times. 105 M-1 cm-1). I partitions from PBS into small unilamellar **liposomes** prepd. from satd. phospholipids and into membranes prepd. from red blood cells (RBC) and binds to human serum albumin (HSA). The I **fluorescence** max. red shifts from 713 nm in PBS to 720-725 nm in **liposomes** and RBC membranes and the **fluorescence** intensity is enhanced 14-35-fold compared to PBS. I is thermally unstable in PBS (half-life = 2 h at 1.3 .times. 10-5M I), but stable in MeOH. In **liposomes** and redblood cell (RBC) membranes, I is 10-fold more stable than in PBS, indicating that it is only partially exposed to the aq. phase. Quenching of I **fluorescence** in **liposomes** and RBC membranes by trinitrobenzene sulfonate also indicates that I is not buried within the membranes. Photodecompn. of I was O-dependent and occurred with a low quantum yield (6.4 .times. 10-4 in PBS). Singlet O was not **detected** upon irradn. of I in membranes or with human serum albumin, since the self-sensitized oxidn. of I occurred at the same rate in D2O as in H2O and was not quenched by NaN3 or histidine.

L46 ANSWER 27 OF 32 MEDLINE DUPLICATE 13

86168232 Document Number: 86168232. The role of cholesterol in the activity of reconstituted Ca-ATPase vesicles containing unsaturated phosphatidylethanolamine. Cheng K H; Lepock J R; Hui S W; Yeagle P L. JOURNAL OF BIOLOGICAL CHEMISTRY, (1986 Apr 15) 261 (11) 5081-7. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The effect of cholesterol on the activity of sarcoplasmic reticulum Ca-ATPase, reconstituted in proteoliposomes containing soybean phosphatidylethanolamine (PE), egg phosphatidylcholine (PC), and cholesterol, was examined. The protein incorporation efficiency increased with PE content but appeared to be independent of cholesterol content. At low cholesterol, PE stimulated calcium uptake. The coupling efficiency of the proteoliposomes increased with an increase in cholesterol content at each PC/(PC + PE) ratio and was more pronounced for those proteoliposomes containing high PE. Dynamic **fluorescence** measurements of the incorporated **lipophilic** probe, diphenyl-1,3,5-hexatriene, revealed a decrease in the motion and an increase in the order of the phospholipid fatty acyl chains in proteoliposomes with high cholesterol content. A complementary observation was made using electron spin resonance of the spin **label**, 2,2-dimethyl-5-dodecyl-5-methyloxazolidine N-oxide. Freeze-fracture electron **microscopy** studies on proteoliposomes containing 0.20 molar ratio of PC/(PC + PE) and cholesterol revealed predominantly vesicular structures with occasional bilayer defects at high cholesterol content. It is postulated that the cholesterol-induced enhancement of the Ca-transport function of the Ca-ATPase is related to the hydration-related bilayer-destabilizing characteristic of the cholesterol molecule as revealed by 31P NMR.

L46 ANSWER 28 OF 32 CAPLUS COPYRIGHT 2000 ACS

1987:403479 Document No. 107:3479 Resonance energy transfer **microscopy**: observations of membrane-bound **fluorescent** probes in model membranes and in living cells. Uster, Paul S.; Pagano, Richard E. (Dep. Embryol., Carnegie Inst. Washington, Baltimore, MD, 21210, USA). J. Cell Biol., 103(4), 1221-34 (English) 1986. CODEN: JCLBA3. ISSN: 0021-9525.

AB A conventional **fluorescence microscope** was modified to observe the sites of resonance energy transfer (RET) between **fluorescent** probes in model membranes and in living cells. These modifications, and the parameters necessary to observe RET between membrane-bound fluorechromes, are detailed for a system that uses N-4-nitrobenzo-2-oxa-1,3-diazole (NBD) or fluorescein as the energy donor and sulforhodamine (SRh) as the energy acceptor. The necessary parameters for RET were first optimized using **liposomes**. Both quenching of the energy donor and sensitized **fluorescence** of the energy acceptor could be directly obsd. in the **microscope**. RET **microscopy** was then used in cultured fibroblasts to identify those intracellular organelles **labeled** by the lipid probe, N-SRh-decylamine (N-SRh-C10). This was done by observing the sites of RET in cells doubly **labeled** with N-SRh-C10 and an NBD-**labeled** lipid previously shown to **label** the endoplasmic reticulum, mitochondria, and nuclear envelope. RET **microscopy** was also used in cells treated with fluorescein-**labeled** Lens culinaris agglutinin and a SRh deriv. of phosphatidylcholine to examine

the internalization of plasma membrane lipid and protein probes. After internalization, the **fluorescent** lectin resided in most, but not all of the intracellular compartments **labeled** by the **fluorescent** lipid, suggesting sorting of the membrane-bound lectin into a subset of internal compartments. RET **microscopy** can colocalize different membrane-bound components at high resoln., and may be particularly useful in examg. temporal and spatial changes in the distribution of fluoroscent mols. in membranes of the living cell.

L46 ANSWER 29 OF 32 MEDLINE DUPLICATE 14

86116714 Document Number: 86116714. Changes in the organization of membrane lipids during human platelet activation. Study by **fluorescent** and freeze-fracture cytochemistry. Lupu F; Calb M; Scurei C; Simionescu N.

LABORATORY INVESTIGATION, (1986 Feb) 54 (2) 136-45. Journal code: KZ4. ISSN: 0023-6837. Pub. country: United States. Language: English.

AB Modifications in the membrane lipid organization of human platelets activated with different agents (adenosine 5'-diphosphate, thrombin, collagen type I, and monosaccharides such as fucose, mannose, and galactose) were analyzed in vitro by using three lipid markers. Cholesterol was **detected** upon interaction with filipin, the anionic phospholipids were reacted with polymyxin B, and alterations in the degree of lipid packing were evaluated with the **lipophilic fluorescent** probe merocyanine 540, which reportedly inserts into bilayer domains whose lipids are more disordered. Filipin-sterol complexes and polymyxin B-anionic phospholipid complexes form characteristic membrane deformations which were examined in freeze-fracture preparations, whereas the merocyanine 540 binding to platelet membrane was recorded by **fluorescent microscopy**. In contrast to the resting cells, thrombin-stimulated platelets displayed an uneven distribution of filipin-sterol complexes which occurred in much higher density on the cell body than on pseudopods: on the latter, apparently cholesterol-free domains were very common. Unlike the non-stimulated cells, the platelets aggregated with the various agents employed showed characteristic polymixin B-anionic phospholipid complexes deformations of plasmalemma suggesting the appearance in uneven concentration of anionic phospholipids in the outer membrane leaflet. Incubation with merocyanine 540 did not result in staining of resting platelets when these were maintained in plasma, but a slight **fluorescence** was observed when platelets were kept in Tyrode buffer. However, platelets stimulated with thrombin, collagen type I, and monosaccharides bound very heavily the **fluorescent** dye; platelets aggregated with adenosine-5'-diphosphate bound only small amounts of merocyanine 540. The results showed that, during activation by different agents, modifications in **lipid membrane** organization include alterations in cholesterol and anionic phospholipid distribution, transbilayer movement of anionic phospholipids accompanied by more disordered membrane.

L46 ANSWER 30 OF 32 MEDLINE DUPLICATE 15

85023285 Document Number: 85023285. Anthracenyl crown ethers and cryptands as **fluorescent** probes for solid-phase transitions of

phosphatidylcholines: syntheses and phospholipid membrane studies. Herrmann U; Tummler B; Maass G; Koo Tze Mew P; Vogtle F. BIOCHEMISTRY, (1984 Aug 28) 23 (18) 4059-67. Journal code: A0G. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Three structurally related crown compounds and cryptands have been synthesized that differ by the number and linkage of coronand units and anthracene moieties. The interaction of the **fluorescent** dyes with sonicated dimyristoylphosphatidylcholine (DMPC) vesicles is characterized by the relative quantum yields, uptake kinetics, binding curves, lifetimes, **fluorescence** titrations with water- and lipid-soluble quenching agents, **fluorescence** anisotropy, and equilibrium cooling curves. The most **lipophilic** compound II, which displays a similar quantum yield as the parent fluorophore 9,10-dimethylanthracene, shows a nearly equal distribution between solid and fluid lipid and is located at the bilayer surface. The least **lipophilic** compound IV is excluded from the hydrocarbon phase. The anthracenophane cryptand III preferentially partitions into solid-phase lecithins with the highest affinity for the phases L epsilon and L beta. The binding constant to DMPC amounts to (5.4 +/- 1.3) X 10(2) M-1 at 0 degrees C. From **fluorescence** quenching titrations it is concluded that the average position of the anthracenoyl dye III discontinuously shifts during the gel to liquid crystalline transition from the glycerol backbone to the choline head group. Electron **microscopy** and NMR experiments revealed that the anthracenophane induces in the liquid crystalline phase the fusion of small unilamellar DMPC vesicles to unilamellar medium-sized vesicles and macrovesicles, which subsequently fuse at the transition temperature to large multilamellar coacervates. Due to its large change of **fluorescence** intensity, the anthracenophane cryptand is a very sensitive probe for the **detection** of the pretransition of symmetrically substituted and of the subtransition of asymmetrically substituted phosphatidylcholines.(ABSTRACT TRUNCATED AT 250 WORDS)

L46 ANSWER 31 OF 32 MEDLINE

83257314 Document Number: 83257314. Melittin induces fusion of unilamellar phospholipid vesicles. Morgan C G; Williamson H; Fuller S; Hudson B. BIOCHIMICA ET BIOPHYSICA ACTA, (1983 Aug 10) 732 (3) 668-74. Journal code: A0W. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Melittin, the soluble **lipophilic** peptide of bee venom, causes fusion of phospholipid vesicles when vesicle suspensions are heated or cooled through their thermal phase transition. Fusion was **detected** using a new photochemical method (Morgan, C.G., Hudson, B. and Wolber, P. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 26-30) which monitors lipid mixing. Electron **microscopy** and gel filtration confirmed that most of the lipid formed large vesicular structures. **Fluorescence** experiments with a water-soluble, membrane-impermeable complex of terbium (Wilschut, J. and Papahadjopoulos, D. (1979) Nature 281, 690-692) demonstrate that these ionic contents are released during fusion. The large structures formed by melittin-induced fusion are impermeable to these ions and are resistant to further fusion. This is in contrast to the behavior observed for the cationic detergent cetyltrimethylammonium bromide (CETAB). The large size of the vesicles formed, the extreme speed of the fusion event and the appearance of electron **microscope** images of the vesicles prior to fusion suggest that the mechanism of the fusion process includes a preaggregation step.

L46 ANSWER 32 OF 32 CAPLUS COPYRIGHT 2000 ACS
1980:442416 Document No. 93:42416 Insertion of **fluorescent** phospholipids into the plasma membrane of a mammalian cell. Struck, Douglas K.; Pagano, Richard E. (Dep. Embryol., Carnegie Inst. Washington, Baltimore, MD, 21210, USA). J. Biol. Chem., 255(11), 5404-10 (English) 1980. CODEN: JBCHA3. ISSN: 0021-9258.

AB Using **fluorescent** derivs. of naturally occurring lipids, low-temp. lipid exchange between small, unilamellar vesicles and cultured Chinese hamster fibroblasts was further investigated, in which vesicle lipids, but not the internal trapped aq. contents, are transferred to recipient cells. Analogs of phosphatidylcholine and phosphatidylethanolamine contg. the **fluorescent** fatty acyl residue, N-4-nitrobenzo-2-oxa-1,3-diazole aminocaaproic acid (NBD-aminocaproic acid), are readily transferred from vesicles to cells at low temp., whereas similar analogs of phosphatidylglycerol and ceramide do not participate in this process. In addn., several phosphatidylethanolamine analogs bearing a fluorophore covalently attached to the polar moiety of the lipid are not transferred to cells via the vesicle-cell exchange process. Apparently, the process of lipid exchange is selective, and the obsd. specificity may be dependent upon the nature of the polar head group of the lipid mol. In cases where lipid exchange was **detected**, examn. of the cells revealed a strong **fluorescent** ring assocd. with the cell periphery, suggesting that the **fluorescent** analog was restricted, primarily, to the plasma membrane of the recipient cells. This was confirmed by the finding that essentially all of the 1-acyl-2-NBD-aminocaproylphosphatidylethanolamine transferred to cells by low-temp. exchange was available for modification by the amino-reactive probe, trinitrobenzene sulfonic acid. In addn., lipids which become cell-assocd. by the exchange mechanism could be quant. transferred from cells to recipient vesicles by a similar process. Finally, using the technique of **fluorescence** recovery after photobleaching, phospholipids incorporated into the fibroblast plasma membrane by vesicle-cell exchange had lateral diffusion rates (2-4 .times. 10-9 cm2/s) which are similar to those obtained using **fluorescent** **lipophilic** dyes. These results are discussed with respect to lipid exchange between serum lipoproteins and cells both in vitro and in vivo.

L47	0	FILE MEDLINE
L48	0	FILE CAPLUS
L49	0	FILE BIOSIS
L50	0	FILE EMBASE
L51	0	FILE WPIDS
L52	0	FILE JICST-EPLUS
L53	0	FILE CONF
L54	0	FILE NTIS

TOTAL FOR ALL FILES

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L55          0 LIPOPHILIC(L) NANOCRYSTAL?(L) FLUORESCEN?

=> s barbera guillem, e?/au,in or guillem e?/au,in

'IN' IS NOT A VALID FIELD CODE
L56         47 FILE MEDLINE
L57         30 FILE CAPLUS
L58         79 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L59         40 FILE EMBASE
L60         19 FILE WPIDS
L61          0 FILE JICST-EPLUS
'AU' IS NOT A VALID FIELD CODE
'IN' IS NOT A VALID FIELD CODE
L62          0 FILE CONF
'IN' IS NOT A VALID FIELD CODE
L63          0 FILE NTIS

TOTAL FOR ALL FILES
L64        215 BARBERA GUILLEM, E?/AU,IN OR GUILLEM E?/AU,IN

=> s l64 and fluorescen?(l)((lipid? or cell)(w)membrane? or liposome?)

L65          0 FILE MEDLINE
L66          0 FILE CAPLUS
L67          0 FILE BIOSIS
L68          0 FILE EMBASE
L69          0 FILE WPIDS
L70          0 FILE JICST-EPLUS
L71          0 FILE CONF
L72          0 FILE NTIS

TOTAL FOR ALL FILES
L73          0 L64 AND FLUORESCEN?(L)((LIPID? OR CELL)(W) MEMBRANE? OR
LIPOSOME
               ?)

=> s l64 and fluorescen?

L74          5 FILE MEDLINE
L75          6 FILE CAPLUS
L76          5 FILE BIOSIS
L77          4 FILE EMBASE
L78          4 FILE WPIDS
L79          0 FILE JICST-EPLUS
L80          0 FILE CONF
L81          0 FILE NTIS

TOTAL FOR ALL FILES
L82         24 L64 AND FLUORESCEN?

=> s l82 not l45

L83          5 FILE MEDLINE
L84          6 FILE CAPLUS
L85          5 FILE BIOSIS
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L86 4 FILE EMBASE
L87 4 FILE WPIDS
L88 0 FILE JICST-EPLUS
L89 0 FILE CONF
L90 0 FILE NTIS

TOTAL FOR ALL FILES
L91 24 L82 NOT L45

=> dup rem l91

DUPLICATE IS NOT AVAILABLE IN 'CONF'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L91
L92 13 DUP REM L91 (11 DUPLICATES REMOVED)

Applicant

=> d cbib abs 1-13

L92 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1
2000:335606 Document No. 133:2215 Signal amplification by crosslinking of nanoparticles by hybridization of bound nucleic acids. **Barbera-Guillem, Emilio**; Nelson, M. Bud; Castro, Stephanie (Biocrystal Limited, USA). PCT Int. Appl. WO 2000028088 A1 20000518, 72 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US26612 19991110. PRIORITY: US 1998-PV107828 19981110; US 1999-437076 19991109.
AB A method of amplifying a signal by crosslinking of water-sol. nanocrystals using nucleic acids immobilized on the particles is described. An anal. probe, e.g. an antibody, attached to one type of particle carrying several mols. of one nucleic acid sequence is used to bind the analyte. This is then incubated with a second type of particle carrying a probe complementary to the nucleic acid on the first type of particle. By alternating incubations with the two types of particles, large branching complexes are built up and easily detected, e.g by fluorometry using a nucleic acid binding dye, or by use of **fluorescent** nanocrystals (quantum dots). Methods of prepg. quantum dots to stabilize them against oxidn. by capping unused reactive groups on the surface are described.

L92 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2
2000:335276 Document No. 132:319310 Functionalized nanocrystals as visual tissue-specific imaging agents, and methods for **fluorescence** imaging. **Barbera-guillem, Emilio** (Biocrystal Limited, USA). PCT Int. Appl. WO 2000027436 A1 20000518, 50 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW,

AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US26591 19991110. PRIORITY: US 1998-PV107828 19981110; US 1999-372729 19990811; US 1999-394635 19990913.

AB Provided is a method of **fluorescence** imaging of living tissue using functionalized nanocrystals. The method comprises contacting an effective amt. of functionalized nanocrystals with the living tissue; exposing the tissue to a spectrum of light suitable for exciting functionalized nanocrystals, present in **fluorescently** labeled tissue, to emit an emission spectrum comprising a **fluorescence** peak; and detecting any **fluorescence** peak emitted by the tissue exposed to the excitation spectrum of light, and obtaining a **fluorescence** image of the tissue. Also provided is a compn. comprising a functionalized nanocrystal which is bound to a substrate in a living tissue, and the use of functionalized nanocrystals in the manuf. of a diagnostic imaging agent.

L92 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3

2000:335218 Document No. 132:331662 Functionalized nanocrystals and their use in detection systems. **Barbera-guillem, Emilio**; Castro, Stephanie (Biocrystal Limited, USA). PCT Int. Appl. WO 2000027365 A1 20000518, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US26487 19991110. PRIORITY: US 1998-PV109626 19981110.

AB Provided are compns. comprising water-sol., functionalized nanocrystals. The water-sol. functionalized nanocrystals comprise quantum dots capped with a layer of a capping compd., and further comprise, by operably linking and in a successive manner, one or more addnl. compds. Preferably, an addnl. compd. comprises diaminocarboxylic acid which is operatively linked to the capping compd., and may further comprise an amino acid, and affinity ligand, or a combination thereof. Also provided are methods of using the functionalized nanocrystals having an affinity ligand to detect the presence or absence of a target substrate in a sample by contacting the functionalized nanocrystals with the sample so that complexes are formed between the functionalized nanocrystals and substrate, if the substrate is present; exposing the complexes in the detection system to an excitation light source, and detecting the emitted **fluorescence** peak. Quantum dot functionalized nanocrystals having a CdSe core, a ZnS shell, and mercaptoacetic acid capping, were activated with EDC and sulfo-NHS and reacted with wheat germ agglutinin. The nanocrystals were then used to **fluorescently** detect Met-129 cancer cells.

L92 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2000 ACS

2000:335682 Document No. 132:354582 Methods for identification and verification. **Barbera-Guillem, Emilio** (Biocrystal Limited,

USA). PCT Int. Appl. WO 2000028598 A1 20000518, 42 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US26615 19991110. PRIORITY: US 1998-PV107829 19981110; US 1999-436145 19991109.

AB Methods of providing a carrier material with an identifiable code pattern are described which entail applying an effective amt. of one or more species of water-sol. nanocrystals by bringing the nanocrystals into contact with the carrier material and linking them to the carrier material. Preferably, the nanocrystals are functionalized nanocrystals (e.g., nanocrystals with coatings chem. modified by materials such as diaminocarboxylic acids to bond to the carrier materials). Code patterns produced from the nanocrystals are also described, as are methods for detecting the patterns which entail exposing a portion of the carrier material to which the identifiable code pattern is applied to an excitation light source and detecting **fluorescence** in the 410-750 nm region emitted by the water-sol. nanocrystals using a detection system which can distinguish between discrete fluoresecence peaks that may be emitted in the spectral range. Application as anticounterfeiting and verification markers is indicated.

L92 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2000 ACS

2000:176014 Document No. 132:205107 Device and process for automated cell staining. **Barbera-Guillem, Emilio**; Olson, Karl (Biocrystal Limited, USA). PCT Int. Appl. WO 2000014534 A1 20000316, 67 pp. DESIGNATED STATES: W: AU, CA, IL, JP, NZ, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20442 19990907. PRIORITY: US 1998-149611 19980908.

AB A cell stainer, for use in an automated cell staining process and from which a fluid contg. stained cells may be dispensed, comprises a fluid flow control means for controlled fluid flow, a plurality of fluid tubing through which is flowed one or more fluids, a chamber means into which is flowed one or more fluids and in which is promoted contact between cells with the one or more fluids contained within the chamber means, and a micro-processor means for controlling the operation of the cell stainer in its performance of an automated cell staining process. Also disclosed is use of the cell stainer in an automated cell staining process comprising a loading phase, a mixing phase, and a dispensing phase; and may further include one or more washing phases. Human lymphocytes were stained using anti-CD5 monoclonal antibody-FITC reagent by the automated cell staining process and by a manual method.

L92 ANSWER 6 OF 13 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 2000-376594 [32] WPIDS

CR 2000-376593 [32]; 2000-387345 [32]; 2000-542942 [32]

AB WO 200028089 A UPAB: 20001006
NOVELTY - Labeled nucleobase (I) comprises a functionalized nanocrystal (A) linked to a nucleobase (NB). (A) and NB each carry one or more reactive functionalities that allow them to be operably linked.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a kit comprising, in separate packages, several different (I), each with a different NB, and differently labeled with a type of (A) that emits **fluorescence**, spectrally resolvable from the **fluorescence** of (A), used to prepare other (I);

(2) a kit comprising, in separate packages, (A) and NB, each with one or more reactive functionalities; and

(3) a method of strand synthesis using (I).

USE - (I) are used for synthesis of nucleic acid strands, e.g. by amplification, reverse transcription, or in vitro mutagenesis, either for labeling or for sequencing, e.g. for use in research, biotechnology, medicine and drug discovery.

ADVANTAGE - (I), which are water-soluble, provide non-isotopic detection, particularly in automated DNA sequencing. Several (I) can be excited with the same light source, providing high quantum yields, and generating spectrally resolvable **fluorescence**, allowing simultaneous detection of several colors, and sequencing in a single reaction. (A) are small enough not to cause steric hindrance.
Dwg.0/3

L92 ANSWER 7 OF 13 BIOSIS COPYRIGHT 2000 BIOSIS
2000:364066 Document No.: PREV200000364066. **Fluorescent** markers for simultaneous detection of multiple labels. Castro, Stephanie L. (1); **Barbera-Guillem, Emilio (1)**. (1) BioCrystal, Ltd., 575 McCorkle Blvd., Westerville, OH, 43082 USA. American Biotechnology Laboratory, (Dec., 1999) Vol. 17, No. 13, pp. 40. print. ISSN: 0749-3223. Language: English. Summary Language: English.

L92 ANSWER 8 OF 13 MEDLINE
95375689 Document Number: 95375689. A simple cell labeling technique by means of lectins linked to fluorochromes for the detection of cells on tissue sections. Alonso-Varona A; Calle Y; Palomares T; Castro B; **Barbera-Guillem E.** (Dpto Biologia Celular y Ciencias Morfologicas, Fac Medicina y Odontologia, Univ del Pais Vasco-Euskal Herriko Unibertsitatea, Barrio Sarriena sn Leioa, Vizcaya, Spain.) BIOLOGY OF THE CELL, (1995) 83 (1) 87-92. Journal code: BOC. ISSN: 0248-4900. Pub. country: France. Language: English.

AB We designed a protocol for cell labeling with the lectin wheat germ agglutinin (WGA) linked to the fluorochrome tetramethyl-rhodamine isothiocyanate (TRITC) for effective detection of the B16F10 melanoma and Lewis lung carcinoma (LLc) cells on pulmonary histological sections from C57BL/6 mice. We have also determined a suitable concentration of WGA-TRITC (10 micrograms/ml), which leads to a very intense and homogeneous labeling of the cells, as it avoids cell clumping due to the presence of the lectin WGA. In order to determine to what extent the method affects these tumor cells, we have studied some important aspects related to their metastatic behavior, taking into account three parameters: a) viability and rate of proliferation of the cells cultured in vitro; b) percentage of animals (C57BL/6 mice) bearing metastasis 15

days after intravenous inoculation with 10(5) B16F10 or LLc cells; and c) pattern of distribution of tumor foci in lung. There were no significant differences in these three parameters between the WGA-TRITC labeled-cells compared to the cultures of non-labeled cells in either of the cell lines (B16F10, LLc). Thus, we conclude that B16F10 and LLc tumor cells can be labeled following the protocol set-up in our study, as it allows these cells to be neatly identified on tissue sections and it causes no important physiological changes in the cells, with regard to metastatic behavior. These points make this technique very suitable for the detection of B16F10 and LLc cells on histological sections in studying their behavior during the first stages of the metastatic process.

L92 ANSWER 9 OF 13 MEDLINE DUPLICATE 4

93339733 Document Number: 93339733. Isolation and enrichment of two sublobular compartment-specific endothelial cell subpopulations from liver sinusoids. Vidal-Vanaclocha F; Rocha M; Asumendi A; **Barbera-Guillem E**. (Department of Cellular Biology and Morphological Sciences, School of Medicine and Dentistry, University of the Basque Country, Vizcaya, Spain..) HEPATOLOGY, (1993 Aug) 18 (2) 328-39. Journal code: GBZ. ISSN: 0270-9139. Pub. country: United States. Language: English.

AB Similar to the well-recognized phenotypical heterogeneity of hepatocytes, in situ sublobular variations have recently been detected in the cell structure, fenestration patterns, filtrating efficiency, surface glycosylation, scavenger function and pathological responses of the sinusoidal lining endothelium. However, unlike other liver cell populations, until now no endothelial cell subpopulations had been isolated or defined with clarity, much less with sublobular/acinar zone-related differential properties. On the basis of our previous studies showing that periportal segments of mouse liver sinusoids express a significantly higher number of wheat germ agglutinin-binding sites than do perivenous ones, we used this differential feature for in vitro labeling of the specific sublobular derivation of isolated sinusoidal lining endothelial cells to correlate their original lobular position with other features determined on flow cytometry, centrifugal elutriation, discontinuous arabinogalactan density gradients and electron microscopy. Our results revealed additional heterogeneous properties whose association with high or low wheat germ agglutinin-binding capacity made it possible to define in vitro two dominant endothelial cell subpopulations that appear similar to the differential features in the periportal and perivenous sinusoidal segments. Type 1 endothelial cells had low forward angle light scatter and high integrated side scatter, low cytoplasmic porosity index (12% +/- 5%) and high wheat germ agglutinin-binding efficiency (160 +/- 35 **fluorescence** intensity units/cell size); these findings are similar to what was observed in situ in the periportal sinusoidal endothelium. On the other hand, type 2 endothelial cells, with high forward angle light scatter and low integrated side scatter, had a high cytoplasmic porosity index (25% +/- 8%) and low wheat germ agglutinin-binding efficiency (60 +/- 15 **fluorescence** intensity units/cell size), findings similar to in situ observations of the perivenous sinusoidal lining endothelium. Moreover, these physical and morphological differences entail different cell sedimentation behaviors;

type 1 endothelial cell sedimented at high centrifugal elutriation counterflow rates (23 to 37 ml/min) and high arabinogalactan density gradient levels (10% to 15%), whereas type 2 endothelial cell sedimented at low counterflow rates (18 to 23 ml/min) and low density levels (6% to 10%). The combination of these separation procedures made it possible to isolate a 90%-enriched type 1 endothelial cell population in the 12% to 15% interphase of the 23 and 37 ml/min elutriation flow rates and a 75%-enriched type 2 endothelial cell population in the 6% to 10% interphase of the 18 and 23 ml/min flow rates.

L92 ANSWER 10 OF 13 MEDLINE DUPLICATE 5

93146652 Document Number: 93146652. Cancer-cell traffic in the liver. II. Arrest, transit and death of B16F10 and M5076 cells in the sinusoids. **Barbera-Guillem E;** Smith I; Weiss L. (Department of Cell Biology and Morphology, University of the Basque Country, Leioa, Spain.) INTERNATIONAL JOURNAL OF CANCER, (1993 Jan 21) 53 (2) 298-301. Journal code: GQU. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB **Fluorescent** probes were used to detect BUdR-labelled B16F10 and M5076 cancer cells delivered to the livers of mice via intrasplenic injection. In liver sections stained for succinic dehydrogenase, which permits the periportal, acinar zone 1 to be distinguished from the pericentral zone 3, counts were made of the zonal distribution of **fluorescent**, intact cancer cells and, by default, the numbers of "lost" cells. Very few intact cancer cells leave the liver from the single bolus of the intrasplenic injection, and even fewer of these generate pulmonary lesions; therefore, within the time limits of these experiments, the liver is virtually a closed system. A dynamic view of intrahepatic cancer-cell traffic with respect to zones 1 (periportal) and 3 (pericentral) was obtained from static measurements of cell densities at different times after intrasplenic injection, by means of Markov chain probability analysis. This indicated that, during the first hour after arrival in zone 1 of the liver sinusoids, there is a 10% probability of a B16F10 cell remaining intact in zone 1, an 89% probability of cell death in zone 1 and only a 1% probability of the cell passing into zone 3. During the same period, there is a 77% probability of an M5076 cell remaining intact in zone 1, a 21% probability of death, and a 2% probability of relocation to zone 3. In both cell types, very few cells were lost from zone 3. Further proportional death in zone 1 diminished over the next 23 hr, concomitant with an increased proportion of cell death in zone 3. Our results indicate that, although there is considerable variation between the 2 cell types studied here, most (B16) or many (M5076) of these cancer cells entering the liver via the portal vein die within 1 hr in zone 1 of liver lobules. In addition, very few of the cells delivered to zone 1 travel along the sinusoids to zone 3, and few of these reach the lungs in a viable state.

L92 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2000 ACS

1993:144024 Document No. 118:144024 Differences in the lectin-binding patterns of the periportal and perivenous endothelial domains in the liver

sinusoids. **Barbera-Guillem, E.**; Rocha, M.; Alvarez, A.; Vidal-Vanaclocha, F. (Sch. Med. Dent., Univ. Basque Country, Vizcaya, 48940, Spain). Cells Hepatic Sinusoid, 3, 199-202 (English) 1991.
CODEN: CHSIEL.
AB Glycoconjugate distribution in liver microvascular endothelium was examd. using a battery of **fluorescently** labeled lectins. Two functional subsets of endothelial cell populations can be distinguished.

L92 ANSWER 12 OF 13 MEDLINE DUPLICATE 6
91293711 Document Number: 91293711. Differences in the lectin-binding patterns of the periportal and perivenous endothelial domains in the liver sinusoids. **Barbera-Guillem E**; Rocha M; Alvarez A; Vidal-Vanaclocha F. (Department of Cell Biology and Morphological Sciences, School of Medicine and Dentistry, University of the Basque Country, Leioa, Vizcaya, Spain..) HEPATOLOGY, (1991 Jul) 14 (1) 131-9. Journal code: GBZ. ISSN: 0270-9139. Pub. country: United States.
Language: English.
AB We have studied the distribution patterns of carbohydrate terminals on the endothelial surface of the mouse liver microvasculature. For this purpose, a wide battery of FITC lectins specific to glucose, mannose, galactose, fucose, N-acetyl-neuraminic acid, N-acetyl-galactosamine and N-acetyl-glucosamine residues were incubated on liver cryostat sections or intraportally perfused under physiological conditions. All the resulting hepatic sections were examined under **fluorescent** microscopy and confocal laser scanning microscopy. With the exception of N-acetyl-galactosamine- and fucose-binding lectins, all the perfused lectins specifically bound to the microvascular wall as confirmed by blocking methods using their corresponding sugars. A wide range of binding was, however, observed among the lectins, and the latter were classified into four groups according to their affinities for the different segments of the hepatic microvasculature: (a) equal affinity for all segments (concanavalin A); (b) different affinities depending on acinar zone (wheat germ agglutinin, Ricinus communis toxin, phytohemagglutinin E, Erythrina cristagalli agglutinin and Pisum sativum agglutinin); (c) preferential binding to the sinusoidal network (Lathyrus odoratus, phytohemagglutinin); and (d) lectins that fail to bind to the hepatic microvasculature (N-acetyl-galactosamine- and fucose-binding lectins). Sinusoidal segment walls in acinar zone 1 expressed a higher concentration of certain lectin-binding carbohydrate residues (N-acetyl-neuraminic acid, N-acetyl-galactosamine, galactose, mannose and glucose) than in acinar zone 3. The labeling patterns obtained through the incubation of liver sections or through in vivo perfusion with the different lectins did not always coincide. Only concanavalin A, wheat germ agglutinin and phytohemagglutinin E lectins proved to be concordant (i.e., they produced identical labeling patterns in both procedures).(ABSTRACT TRUNCATED AT 250 WORDS)

L92 ANSWER 13 OF 13 MEDLINE DUPLICATE 7
90142522 Document Number: 90142522. Functional variations in liver tissue during the implantation process of metastatic tumour cells.
Vidal-Vanaclocha F; Alonso-Varona A; Ayala R; **Barbera-Guillem E.**
(Department of Cell Biology and Morphological Sciences, School of Medicine, University of the Basque Country, Vizcaya, Spain.) VIRCHOWS ARCHIV. A, PATHOLOGICAL ANATOMY AND HISTOPATHOLOGY, (1990) 416 (3) 189-95.
Journal code: XD1. ISSN: 0174-7398. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB We have examined several properties of sinusoidal cells in the unaffected tissue of micrometastasis-containing livers. Tumour cells from either B16 melanoma (B16F10) or Lewis lung carcinoma (LLC) were injected intrasplenically in syngeneic mice and sacrificed on the 7th day. Light and scanning electron microscopy (SEM) showed tumour cells in hepatic veins and sinusoids in close contact with endothelial walls and macrophages. Following quantitative analysis of SEM images from sinusoidal walls it was found that endothelial fenestrae from B16F10 or LLC-colonized livers were diffusely reduced both in size and density/microns 2 throughout the sinusoid wall, although especially affected zone 3 segments. Following the intrasplenic injection of 1 microns **fluorescent** latex particles 1 h prior to sacrifice of the mice a significant reduction of the latex particle uptake by sinusoidal cells was detected in B16F10-colonized livers (27% of controls) which was in contrast to the significant increase in LLC-colonized mice (180% of controls). Despite the focal character of the tumour cell implantation process, hepatic sinusoidal cells reacted diffusely to metastatic cells. However, over liver acini, endothelial cell changes were mainly expressed in zone 3 while phagocytic properties mainly varied in zone 1 and depending on the tumour type. Although the significance of these sinusoidal changes on metastatic development is unclear, data suggests that "soil" conditions in the liver are different before and after being metastasized by tumour cells.

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